Environmental Health Perspectives
Vol. 21, pp. 211–214, 1977

Effects of Subanesthetic Concentrations of Enflurane on Rat Pregnancy and Early Development

by C. Denny Strout,* Michael L. Nahrwold,* Margaret D. Taylor,* and Ian S. Zagon*

Anesthetic pollutants in the operating room have been implicated in producing spontaneous abortion in exposed personnel and congenital malformations among their offspring. To test the effects of trace concentrations of enflurane on pregnancy, rats were exposed to two levels (10.7 and 63.7 ppm) of the anesthetic for 8 hr daily from days 1 to 19 of pregnancy. Litter sizes were not affected but birth weights of exposed offspring were slightly higher than controls. During lactation, cross-fostering studies were performed. Exposed offspring were housed with nonexposed mothers and vice versa to determine if exposure during pregnancy affected early development. Weights at 7, 14, and 21 days of age did not differ among the offspring in the lower dose experiment. Weights of the cross-fostered groups in the high dose experiment were decreased at day seven compared to controls. In the same experiment, exposed offspring housed with exposed mothers were heavier than controls on day 21 of lactation. The modest nature of these alterations suggests that enflurane has little or no gross effect on rat pregnancy and postnatal development.

The effects of anesthetic pollutants on operating room personnel have been the subject of increasing interest as set forth in a recent review (*1*). Trace levels of anesthetics have been implicated in producing an increased incidence of cancer (*2, 3*), liver disease, and kidney disease (*2*). A potential hazard of great concern involves chronic exposure to trace anesthetics during pregnancy. An increased incidence of spontaneous abortion among anesthetists and other operating room personnel has been reported by several authors (*2, 4–7*). However, a cause–effect relationship has not been established and there is some evidence that factors other than anesthetics, such as stress (*7*), may be responsible. Nevertheless, there have been reports of an increase in the incidence of congenital malformations among the offspring of anesthetists (*2, 6, 8*).

Although embryolethality has been demonstrated in laboratory animals exposed to high concentrations of anesthetics (*9–11*), there is little evidence that levels commonly found in the operating room are also embryolethal. Corbett et al. reported an increased incidence of fetal deaths in rats exposed to 1,000 and 15,000 ppm of nitrous oxide during the mid and late gestation periods (*12*). However, 100 ppm did not have a significant effect. Bruce showed that exposure of mice to 16 ppm of halothane prior to and during pregnancy neither affected fertility of males or females nor did it affect the outcome of pregnancy (*13*). Similar findings were reported by Wharton et al. in mice exposed to 500 and 1000 ppm of halothane (*14*). Corbett et al. found no embryolethality for 100 ppm of halothane or 100 ppm plus 0.5% nitrous oxide in rats exposed during mid-pregnancy (*15*).

In recent years, enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether), a volatile anesthetic, has gained popularity in this country. There have been no published reports to date on the effects of trace levels of this agent on pregnancy. Therefore, we have exposed pregnant rats to two concentrations of this anesthetic under simulated operating room conditions. Weight gain of the mothers during pregnancy, litter size, and birth weight of the offspring were utilized as indices of fetal development. Weight gain of the pups in cross-fostering experiments was measured in an effort to detect developmental abnormalities.

*Departments of Anesthesiology and Anatomy, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033.

Methods

Female, virgin Sprague-Dawley rats of the same approximate age and weight were obtained from Charles River Breeders and mated. Day 1 of pregnancy was diagnosed with the presence of vaginal sperm. The animals were then randomly assigned to either an experimental or a control group. Two experiments were performed, with low and high dose enflurane exposure for 8 hr/day (8:00 a.m. to 4:00 p.m.) from day 1 to day 19 of pregnancy. A diurnal cycle of alternating 12-hr periods of light and dark was maintained, with exposure occurring during the light period.

During the exposure period the rats were housed, five to six animals per cage, in standard 38 × 46 × 20 cm mesh-bottom cages and provided with free access to food and water. Isolation cubicles measuring 1.83 × 3.15 × 1.22 m and constructed of painted cinderblock with sliding Plexiglas front panels served as the exposure chambers. The control and experimental cubicles were identical but in separate rooms. Room air, which had been filtered through Fiberglass and charcoal, was drawn through the bottom of the cubicle and exhausted through the top to provide an air turnover rate of 18 cycles/hr. This was adequate to maintain carbon dioxide at less than 0.1% as measured by infrared analysis, and oxygen at normal ambient levels as measured by paramagnetic analysis. Enflurane was delivered into the experimental chamber in filtered compressed air via a Dräger vaporizer. Air samples were withdrawn from each cage several times daily via Teflon tubing and analyzed for enflurane concentration by gas chromatography. Temperature and humidity within the cubicle were similarly monitored. Figures 1 and 2 show the conditions within the cubicles on a daily basis for each of the experiments. During the low-dose experiment, mean enflurane concentration was 10.7 ppm (S.D. = 1.4), while in the high-dose experiment it was 63.7 ppm (S.D. = 13.6). All animals were weighed every other day. No differences were noted in the pattern of weight gain, feeding habits, behavior, or appearance between the experimental and control groups.

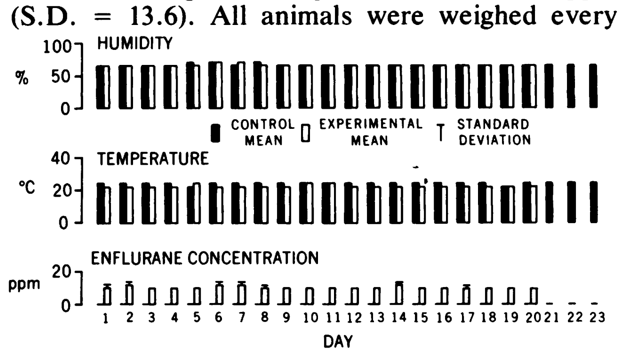

FIGURE 1. Cubicle conditions (low dose).

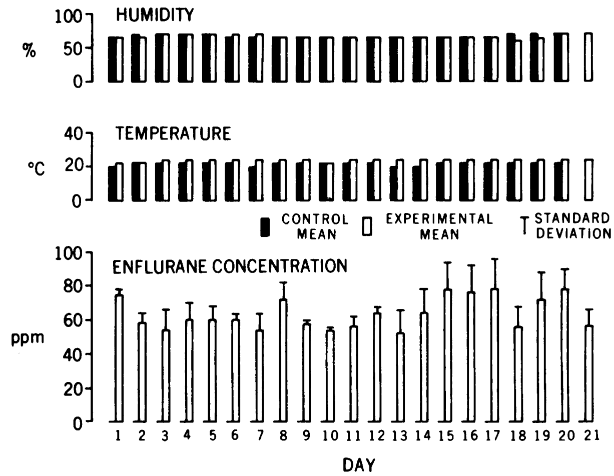

FIGURE 2. Cubicle conditions (high dose).

At the end of the nineteenth day of gestation, the rats were removed from the cubicles and placed individually in solid bottom cages to deliver their offspring. The day of gestation on which birth had occurred, total number of live births, and the birth weights of the young were recorded. The uteri of those animals which did not deliver were examined. None showed signs of pregnancy and those rats were eliminated from the study.

During lactation, a cross-fostering study was performed for both the low- and high-dose experiments. Each of three experimental (enflurane-exposed) mothers were caged separately with eight control (nonexposed) offspring, and each of three control mothers were caged with eight experimental offspring. In addition, three each of the experimental and control mothers were caged with eight offspring of their own group. Weights of the offspring were recorded at 7, 14, and 21 days of age.

Results

The mean weight of mothers in the control and experimental groups did not differ at day 1 or day 19 of gestation during both the low-dose and high-dose experiments (Table 1). Similarly, control and experimental litter sizes did not differ in either experiment (Table 2). Birth weights of enflurane-exposed offspring were slightly higher than the nonexposed controls in each of the two experiments (Table 3).

There were no weight differences among the offspring during lactation in the low-dose study at 7, 14, and 21 days (Table 4). However, in the high-dose experiment, the two cross-fostered groups weighed less at 7 days when compared to control mothers with control offspring (Table 5). These differences were not apparent at 14 days. However, at 21 days experimental pups housed with experimental mothers weighed significantly more than control mothers housed with control offspring.

Table 1. Weight gain during gestation.

Day of gestation	Experiment	Mean weight ± SD, g Control	Experimental
1	Low dose	209 ± 4.9 (n = 9)	203 ± 7.7[a] (n = 9)
19	Low dose	323 ± 7.4 (n = 9)	317 ± 10.0[a] (n = 9)
1	High dose	214 ± 14.5 (n = 6)	208 ± 18.9[a] (n = 13)
19	High dose	334 ± 18.9 (n = 6)	330 ± 33.9[a] (n = 13)

[a]No different from control by Student's t-test for unpaired data.

Table 2. Litter size.

	Mean litter size ± SD (range)		Number of litters	
Experiment	Control	Experimental	Control	Experimental
Low dose	13.8 ± 2.1 (11–17)	11.7 ± 2.3[a] (6–14)[b]	9	9
High dose	11.7 ± 1.0 (10–13)	11.5 ± 1.9[a] (7–14)	6	13

[a]No different from control by Student's t-test for unpaired data.
[b]Range is 11–14 excluding one litter of 6.

Table 3. Birth weights of offspring.

Experiment	Group	Mean weight ± SD, g	n
Low dose	Control	5.93 ± 0.39	124
Low dose	Experimental	6.18 ± 0.51*	105
High dose	Control	6.53 ± 0.61	70
High dose	Experimental	6.74 ± 0.74*	150

[a]Differs from control, $p < 0.01$, by Student's t-test for unpaired data.

Discussion

An attempt was made to simulate the operating room environment and the conditions under which operating room personnel are exposed to anesthetics. The two doses of enflurane were chosen to approximate the range of values commonly measured in the area of the anesthetist in our operating room. A daily 8-hr exposure period was chosen to simulate a normal working day. Enflurane exposure was started on day 1 of gestation so that any early effects on pregnancy would be detected.

Our data suggest that enflurane, in the concentrations tested, has little or no gross effect on either rat pregnancy or early postnatal development. The smallest litter among the exposed rats was six offspring. Examination of the uterus of this rat revealed placental scars in only one horn of the bicornuate uterus. This may have been due to an isolated anatomical defect. The differences found in the birth weights could reflect a modest anesthetic effect or a variation in gestational age. The weight decrement found at day seven in both cross-fostered groups of the high-dose experiment did not persist to day fourteen. Exposed offspring fostered by exposed mothers actually weighed more than controls on day 21 in the same experiment. Therefore, it is difficult to postulate either maternal or fetal effects of subanesthetic enflurane exposure from these findings.

We conclude that, under the conditions of this experiment, enflurane has no gross effects on rat pregnancy or early postnatal development.

Table 4. Weights of offspring during lactation: low-dose experiment.

Groups Mothers	Offspring	Mean weights ± SD, g 7 days	14 days	21 days
Control	Control	16.0 ± 0.9 (n = 24)	30.1 ± 1.3 (n = 24)	45.8 ± 3.3 (n = 24)
Control	Experimental	15.2 ± 1.3 (n = 24)	29.0 ± 2.4 (n = 23)	44.8 ± 4.2 (n = 23)
Experimental	Control	15.7 ± 1.3 (n = 24)	30.6 ± 2.4 (n = 22)	45.2 ± 4.0 (n = 22)
Experimental	Experimental	15.7 ± 2.9 (n = 24)	28.4 ± 5.8 (n = 24)	45.4 ± 8.3 (n = 24)

Table 5. Weights of offspring during lactation: high dose experiment.

Groups Mothers	Offspring	Mean weights ± SD, g 7 days	14 days	21 days
Control	Control	18.9 ±1.0 (n = 24)	34.6 ± 1.8 (n = 24)	52.7 ± 7.6 (n = 24)
Control	Experimental	16.3 ± 2.1[a] (n = 24)	34.7 ± 4.1 (n = 24)	55.4 ± 8.3 (n = 24)
Experimental	Control	16.9 ± 2.6* (n = 24)	33.4 ± 4.4 (n = 24)	54.6 ± 7.4 (n = 24)
Experimental	Experimental	18.1 ± 1.9 (n = 24)	34.8 ± 2.4 (n = 24)	59.9 ± 4.6* (n = 24)

[a]Different from control mothers with control offspring ($p < 0.01$) when tested with analysis of variance followed by Dunnett's procedure (*16*).

REFERENCES

1. Fink, B. R., and Cullen, B. F. Anesthetic pollution: what is happening to us? Anesthesiology 45: 79 (1976).
2. Cohen, E. N., et al. Occupational disease among operating room personnel: a national study. Report of an ad hoc committee on the effect of trace anesthetics on the health of operating room personnel, American Society of Anesthesiologists. Anesthesiology 41: 321 (1974).
3. Bruce, D. L., et al. Causes of death among anesthesiologists: a 20-year survey. Anesthesiology 29: 565 (1968).
4. Askrog, V., and Harvald, B. Teratogen effekt af inhalationsanaestetika. Nord. Med. 83: 498 (1970).
5. Cohen, E. N., Bellville, J. W., and Brown, B. W. Anesthesia, pregnancy, and miscarriage: a study of operating room nurses and anesthetists. Anesthesiology 35: 343 (1971).
6. Knill-Jones, R. P., et al. Anaesthetic practice and pregnancy: controlled survey of women anaesthetists in the United Kingdom. Lancet 1: 1326 (1972).
7. Rosenberg, P., and Kirves, A. Miscarriages among operating theatre staff. Acta Anaesthesiol. Scand. (Suppl.) 53: 37 (1973).
8. Corbett, T. H., et al. Birth defects among children of nurse-anesthetists. Anesthesiology 41: 341 (1974).
9. Fink, B. R., Shepard, T. H., and Blandau, R. J. Teratogenic activity of nitrous oxide. Nature 214: 146 (1967).
10. Basford, A. B., and Fink, B. R. The teratogenicity of halothane in the rat. Anesthesiology 29: 1167 (1968).
11. Bussard, D. A., et al. Fetal changes in hamsters anesthetized with nitrous oxide and halothane. Anesthesiology 41: 275 (1974).
12. Corbett, T. H., et al. Effects of low concentrations of nitrous oxide on rat pregnancy. Anesthesiology 39: 299 (1973).
13. Bruce, D. L. Murine fertility unaffected by traces of halothane. Anesthesiology 38: 473 (1973).
14. Wharton, R. S., et al. Fertility and embryolethality in mice chronically exposed to halothane. In: Abstracts of Scientific Papers, 1976 Annual Meeting, American Society of Anesthesiologists, p. 143.
15. Corbett, T. H., et al. Teratogenicity studies with halothane (100 ppm) and halothane (100 ppm) plus nitrous oxide (0.5%). In: Abstracts of Scientific Papers, 1976 Annual Meeting, American Society of Anesthesiologists, p. 139.
16. Dunnett, C. W. A multiple comparison procedure for comparing several treatments with a control. Amer. Statist. Assoc. J. 50: 1096 (1955).